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BIOCHEMICAL COMPOSITION AND FATTY ACID PROFILE OF THE MARINE MICROALGA *Isochrysis galbana* DRIED WITH DIFFERENT METHODS

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ABSTRACT

In this paper, the effects of different drying methods on proximate composition, pigment contents, and fatty acids profile of *Isochrysis galbana* were evaluated. The microalgae biomass was dried by freeze drying (FD), oven drying (OD), spray drying (SPD), and sun drying (SD) methods to identify the best procedure with the lowest negative impacts on the algae nutrient values including pigments, proximate composition, and fatty acid profile. The highest protein content was obtained in FD (62.20±0.15 %). Also, the lipid content of the dried microalga was significantly influenced by the drying process, while among all treatments, the highest and lowest values were measured in FD (13.77±0.42 %) and SD (11.68±0.16 %) samples, respectively (P<0.05). The highest chlorophyll *a* (0.902±0.028 µg/mg), *b* (0.605±0.007 µg/mg), and total carotenoids (1.057±0.056 µg/mg) were observed in FD samples. However, the lowest levels of chlorophylls and total carotenoid pigments were obtained in SD and SPD, respectively. The lipid profiling analysis showed the highest level of polyunsaturated fatty acids (PUFAs) in FD method (41.51%), while the maximum saturated fatty acids were observed in SD (54.89%) followed by OD (51.41%). Also, the highest docosahexaenoic acid (12.41 %) were measured in FD compared to others (P<0.05). In conclusion, freeze drying method would be an efficient dewatering post-harvesting technique for the marine microalga *I. galbana* with the lowest impact on the nutritional quality in particular PUFAs content compared to the other studied drying methods.

Keywords: Carotenoids, Drying methods, Fatty acids, *Isochrysis galbana*

INTRODUCTION

Microalga with a simple structure uses carbon dioxide and dissolved nutrient ions in water via sunlight for synthesis of different organic materials like pigments and other bioactive compounds **(Pokoo-Aikins *et al*., 2010; Sabzi *et al*., 2018; Rastar *et al*., 2018)**. These microorganisms contain potential source of polyunsaturated fatty acids (PUFAs), and have been known as a promising renewable source of biofuel due to their high growth rate, low land usage and relatively high extractable oil yield **(Chisti, 2007; Brennan and Owende, 2010; DalmasNeto *et al*., 2014; Sabzi *et al*., 2018)**. Furthermore, microalgae have been widely cultured and utilized as a live food in aquaculture industry **(Welladsen *et al*., 2014; Rastar *et al*., 2018)**.

Due to their unique nutritional values and health benefits, microalgae species are also being reconsidered in human diets **(Chacón-Lee and González-Mariño, 2010, Vigani *et al*., 2015; FAO, 2016; Sabzi *et al*., 2018)**. However, ways of harvesting and dewatering of microalgal biomass are critical issues associated with the recovering of the nutritional components. Several commercial techniques including freeze, spray, and drum dryers of microalgae cultures have been employed **(Chua and Chou, 2003; Grima *et al*., 2013; Chen *et al*., 2015)**. Among the various methods of drying, solar is commonly used procedure because of low cost and energy demands **(Mata *et al*., 2010)**. However, this method may cause severe deterioration and denaturation of the valuable microalgae bio-products due to high microbial and biochemical spoilage rates during the drying process **(Kumar *et al*., 2010; Grima *et al*., 2013; Stramarkou *et al*., 2017)**. In contrast, the freeze drying method has been shown to be the best technique due to high lipid yield and biodiesel quality of *C. vulgaris* **(Hussain *et al*., 2015)**.

Marine microalgae, *Isochrysis galbana* is the best-known source of some essential nutrients, particularly PUFAs and is one of the most common species used for fish and shellfish larvae culture **(Lin *et al*., 2007; Guihéneuf *et al*., 2009)**. There is little literature comparing the effects of different drying methods on microalgae nutritional value. Therefore, this study attempt to compare the effects of four different drying methods i.e. spray, oven, solar and freeze drying on nutritional composition and fatty acids profile of *I. galbana*.

MATERIALS AND METHODS

Algae culture

The initial purified seed of *I. galbana* was obtained from the Persian Gulf Biotechnology Park (Gheshm, Iran) and transported at Zakarya-e-Razi Laboratory Complex, IAU University (Tehran, Iran). The algae was grown in f2 medium **(Guillard and Ryther, 1962)** under culture conditions: 3511±351 IL luminance (Lux) by fluorescent lamps, pH 8, temperature 20±2 °C, and 2.5 % salinity. After 14 days of culture, the algae was harvested by using a Sigma 3-30KS centrifuge (Osterode, Germany) at 7100 ×g for 15 min at 4°C.

Drying methods

The fresh microalgal biomass (120 L) was dewatered using four different drying techniques: sun dried (SD) at 22-36 °C for 2 days, freeze dried (FD) at -84°C under high vacuum conditions (0.04 mbar) for 12 h by a Christ Alpha 1–4 freeze dryer (Christ, Germany), oven dried (OD) at 60 °C for 12 h, and spray dried (SPD) (liquid suspension of algae) through a Buchi B-191 spray dryer (Buchi Laboratoriums-Technik AG, Switzerland) at 140-150 °C and 80-85 °C as the inlet and outlet temperatures, respectively for 6-8 seconds.

Chlorophyll *a*, *b* and carotenoid contents

Chlorophyll (Chl) contents of each treatment was determined using method described by **Yang *et al*. (1998)**. Briefly, the dried samples (10 mg) were mixed with 5 mL of 80% acetone in a vortex for 5 min, centrifuged at 1500 ×g for 5 min, and the supernatant was collected. Chl *a*, *b* and total carotenoid levels were measured using a Cary 50 UV-Vis spectrophotometer (Varian Inc., USA) at 663, 645 and 470 nm, respectively (Porra et al. 1989; Holm 1954). The Chl *a*, *b* and carotenoids contents were calculated by the following equations:

$$\text{Chl } a\ (\mu g/mL) = 12.25 \times A_{663} - 2.25 \times A_{645}$$
$$\text{Chl } b\ (\mu g/mL) = 20.31 \times A_{645} - 2.25 \times A_{663}$$

$$\text{Total carotenoids } \left(^{\mu g}/_{mL}\right) = (\text{Chl } a + \text{Chl } b) + \frac{(1000 \times A_{470}) - (1.90 \times \text{Chl } a) - (63.14 \times \text{Chl } b)}{214}$$

Where, A is the optical density (OD) by the spectrophotometer.

Proximate composition

Residual moisture in each sample (1 g) was determined through an automatic moisture analyzer (Sartorius MA30, Germany) at 105ºC for up to 60 min until reached a constant weight of the sample. Total protein was calculated (N×6.25) by the Kjeldahl method **(AOAC, 2000)**. The extraction of total lipids was carried out in accordance with **Bligh and Dyer (1959)** method. Total ash content was calculated by a muffle furnace at 550 °C for 8 h.

Fatty acids profile

Fatty acids (FAs) composition of different dried samples were measured according to **Cohen *et al*. (1993)** method and the results were expressed as % of total fatty acids (TFAs). FAs were trans esterified in 2% H_2SO_4-methanol solution at 80°C for 1 h, followed by adding n-heptane to the mixture prior to stirring and centrifugation at 2150 ×g for 10 min. Fatty acid methyl esters (FAMEs) were analyzed by a Younglin ACME 6100 Gas Chromatograph (Anyang, Korea) equipped with a mass selective detector (Dikmacap-2330), a capillary column DB-225JW (30 m × 0.25 mm × 0.25 µm), and helium used as the carrier gas with a flow rate of 2.6 mL/min. The injection temperature, ion temperature and interface temperature were set at 250, 200 and 260 °C, respectively, and the split ratio was 1:100.

Statistical analysis

All measurements were carried out in triplicates and the mean data and their standard errors were obtained. All variances were checked for normality and homogeneity, and one-way analysis of variance (ANOVA) was used to determine the significant difference in the dependent variables. Post-hoc Tukey-test at reliability level of 5% was used to identify differences between each level of treatment.

RESULTS

Changes in the biochemical composition

Results of the proximate biochemical composition are summarized in Table 1. The highest protein (62.20±0.15 %) and moisture (6.46±0.134 %) contents were determined in FD and SD treatments, respectively. The Lipid content was significantly elevated in SPD samples (P<0.05). Also, minimum level of lipid content (17.89±0. 12%) was observed in FD samples. The highest and lowest ash content were obtained in FD (13.77±0.42 %) and SD (11.68±0.16%), respectively.

Table 1 Proximate composition (% in dry weight) of dehydrated *Isochrysis galbana* by different drying methods. OD: oven dried, FD: freeze dried, SD: sun dried, and SPD: spray dried.

Parameters (%)	Drying methods			
	OD	FD	SPD	SD
Moisture	3.94±0.29 ^{b}	4.03±0.14 ^{b}	4.10±0.22 ^{b}	6.46±0.134^{a}
Protein	60.08±0.53^{b}	62.20±0.15^{a}	60.53±0.38^{b}	60.10±0.41^{b}
Lipid	19.13±0.09^{b}	17.89±0. 12^{c}	20.72±0.22^{a}	19.19±0.18^{b}
Ash	12.83±0.52^{b}	13.77±0.42^{a}	12.38±0.37^{b}	11.68±0.16^{c}

Mean values (±standard error) followed by different letters in the same raw indicate a statistical difference (n=3, p < 0.05).

Changes in the pigment contents

As shown in Table 2, the highest content of Chl *a* was obtained in FD (0.902±0.028 µg/mg) compared to other treatments (P<0.05). Also, the highest Chl b was measured in FD (0.605±0.007 µg/mg), while the minimum value was obtained in SD (0.490±0.004 µg/mg). Further, the highest total carotenoid was obtained in FD (1.057±0.056 µg/mg), while SPD samples showed the minimum value (0.735±0.028 µg/mg) (P<0.05).

Table 2 Pigment contents (µg/mg in dry weight) of dehydrated *Isochrysis galbana* by different drying methods. OD: oven dried, FD: freeze dried, SD: sun dried, and SPD: spray dried.

Pigments	Drying methods			
	OD	FD	SPD	SD
Chlorophyll *a*	0.738±0.004^{bc}	0.902±0.028^{a}	0.776±0.014^{b}	0.701±0.007^{c}
Chlorophyll *b*	0.510±0.002^{b}	0.605±0.007^{a}	0.507±0.003^{b}	0.490±0.004^{c}
Total carotenoids	0.844±0.049^{b}	1.057±0.056^{a}	0.735±0.028^{c}	0.849±0.014^{b}

Mean values (±standard error) followed by different letters in the same raw indicate a statistical difference (n=3, p < 0.05).

Table 3 Fatty acids profile (% of total fatty acid) of dehydrated *Isochrysis galbana* by different drying methods. OD: oven dried, FD: freeze dried, SD: sun dried, and SPD: spray dried.

Fatty acids		Drying methods			
		OD	FD	SPD	SD
C8:0	Caprylic acid	0.18±0.01^{d}	0.51±0.02^{b}	1.00±0.03^{a}	0.27±0.02^{c}
C10:0	Capric acid	2.59±0.09^{b}	3.88±0.01^{a}	3.87±0.32^{a}	2.83±0.06^{b}
C12:0	Lauric acid	1.47±0.08^{c}	0.9±0.01^{d}	2.82±0.04^{a}	1.75±0.09^{b}
C14:0	Myristic acid	16.91±0.14^{a}	16.03±0.12^{b}	16.88±0.18^{a}	16.90±0.04^{a}
C15:0	Pentadecylic acid	1.88±0.01^{b}	1.27±0.01^{c}	1.98±0.16^{a}	1.91±0.01^{a}
C16:0	Palmitic acid	21.93±0.15^{a}	18.13±0.32^{b}	21.99±0.03^{a}	21.98±0.22^{a}
C17:0	Margaric acid	0.15±0.04^{c}	0.32±0.01^{b}	0.48±0.01^{a}	0.50±0.05^{a}
C18:0	Stearic acid	1.06±0.01^{b}	0.91±0.04^{b}	2.48±0.33^{a}	1.05±0.01^{b}
C20:0	Arachidic acid	0.06±0.02^{c}	0.03±0.01^{c}	0.69±0.05^{a}	0.55±0.03^{b}
C21:0	Heneicosylic acid	1.19±0.05^{a}	0.08±0.01^{b}	0.07±0.02^{b}	1.09±0.01^{a}
C22:0	Behenic acid	0.05±0.00^{b}	0.04±0.00^{b}	0.08±0.00^{a}	0.06±0.00^{a}
Σ SFAs		52.41	42.10	52.34	54.89
C14:1	Myristoleic acid	0.18±0.01^{c}	0.49±0.06^{a}	0.38±0.05^{b}	0.45±0.04^{ab}
C16:1	Palmitoleic acid	3.63±0.06^{c}	7.76±0.05^{a}	2.79±0.17^{d}	5.55±0.19^{b}
C17:1	Heptadecenoc acid	0.74±0.03^{b}	0.83±0.09^{a}	0.59±0.07^{c}	0.25±0.04^{d}
C20:1	Eicosenoic acid	0.72±0.02^{b}	0.34±0.01^{d}	0.96±0.07^{a}	0.5±0.05^{c}
C24:1	Nervonic acid	0.037±0.01^{b}	0.05±0.01^{a}	0.04±0.01^{ab}	0.01±0.01^{c}
C18:1n-1	Vaccenic acid	1.52±0.1^{b}	1.2±0.01^{bc}	2.5±0.4^{a}	1.04±0.01^{c}
C18:1n-9	Oleic acid	12.31±0.04^{c}	16.50±0.09^{a}	15.13±0.17^{b}	11.51±0.06^{d}
Σ MUFAs		16.32	20.98	19.39	16.31
C18:2	Linoleic acid	9.27±0.12^{c}	11.94±0.2^{a}	10.07±0.53^{b}	9.87±0.13^{b}
C18:4	Stearidonic acid	9.13±0.22^{c}	14.41±0.3^{a}	9.68±0.29^{b}	9.08±0.03^{c}
C20:5	Eicosapentaenoic acid	1.06±0.00^{c}	2.75±0.04^{a}	2.06±0.02^{b}	1.05±0.00^{c}
C22:6	Docosahexaenoic acid	8.12±0.07^{c}	12.41±0.08^{a}	9.9±0.06^{b}	8.52±0.25^{c}
Σ PUFAs		27.58	41.51	31.71	28.52

Mean values (±standard error) followed by different letters in the same raw indicate a statistical difference (n=3, p < 0.05).

Changes in fatty acids compositions

FAs composition of dewatered *I. galbana* by different drying procedures is given in Table 3. Myristic acid (14:0) and palmitic acid (C16:0) were the most abundant saturated fatty acids (SFAs) which were the highest values in all treatments except for FD samples. Palmitoleic acid (C16:1) and oleic acid (C18:1) were mainly proportions of MUFAs, and the highest values was measured in FD treatment (7.76±0.05 % of TFAs and 16.50±0.09 % of TFAs, respectively). Linoleic acid (C18:2), stearidonic acid (C18:4) and docosahexaenoic acid (DHA) were the major fatty acids in PUFAs component. Also, the highest linoleic acid (11.94±0.20 % of TFAs), stearidonic acid (14.41±0.30 % of TFAs), and DHA (12.41±0.08 % of TFAs) contents were obtain in FD samples compared to other treatments ($P<0.05$).

DISCUSSION

The results indicated that the highest protein content was obtained in FD method compared to other methods. The reason why a higher protein level was seen in FD samples compared to hot-air drying methods might be in part due to the loss of numerous nitrogen content with some volatile nitrogen based compounds which can leads to reducing of crude proteins content. However, FD probably preserve the protein content of microorganisms against direct heating. **Stramarkou *et al*. (2017)** reported higher content of protein and pigments in freeze-dried of *C. vulgaris* compared to hot-air dried samples. Furthermore, **Desmorieux and Hernandez (2004)** studied on the influence of different drying processes (convective, infrared drying, spray drying, and freeze drying) and compared proximate composition of dried *Spirulina*. Their results revealed that Freeze-drying showed the lowest protein.

Our results also showed that the moisture content in all treatments was less than 10% with the SD method showed the highest level. Therefore, it can be illustrated by high efficiency of drying methods in this study to dehydrate the algae cells.

The highest lipid content was measured in SPD followed by SD and OD samples, while the lowest amount of lipid was obtained by FD method. In a study by **Hussain *et al*. (2015)** the highest lipid level in *C. vulgaris* using OD drying method compared to FD methods due to more cell disruptions during oven drying period. Mass-transfer through the solid matrix is usually the rate-controlling step in extraction; and because of the lowest impact on demolishing of the microorganism's cell walls in FD method, the resistance to oil diffusion is higher than hot-air drying methods **(Aguilera and Stanley, 1999; Gutiérrez *et al*., 2008)**. Similarly, **Ryckebosch *et al*. (2012)** indicated negligible impact of freeze-drying on total lipids contents and FAMEs composition of *Phaeodactylum tricornutum* when it was compared with the fresh samples.

Total carotenoid and chlorophylls contents are important groups of primary metabolites in microalgae species which are highly affected by heating temperature and duration. FD method preserved a high level of Chl *a*, *b* and carotenoids contents in *I. galbana* biomass after dewatering. A significant loss can be happened when the carotenoids are exposed to over 50°C. Some investigators reported that algae species usually loss their crucial essential nutrients such as carotenoids and chlorophylls during the dewatering process **(Dey and Rathod, 2013)**. A marked reduction in total carotenoids of dewatered *I. galbana* was found in SPD due to the highest temperature in the drying process. Also, **Ryckebosch *et al*. (2011)** reported that the carotenoid content of the spray-dried microalga was significantly lower than that of the fresh and freeze-dried algae. This reduction may be explained by thermal breakdown and complexation of destroying carotenoids **(Tang *et al*., 2000)**.

Hot-air drying usually destroys the cell structures, however, the primary structure and the shape of the microorganism's structures relatively stay protected in FD due to formation of the ice crystal inside the tissue matrix and the water get directly out from the solid to the gas phase, without any heating process **(Youssef and Mokhtar, 2014)**. Also, heating process in conventionally dried techniques leads to accelerate the oxidation process of the dried microalgae biomass which can adversely impact on nutritional values and color of the final products **(An-Erl King *et al*., 2001; Güroy *et al*., 2017)**. **Hsu *et al*. (2003)** found that freeze-drying method can preserve antioxidant activity of *Dioscorea alata* and *D. purpurea*. Also, **Güroy *et al*. (2017)** stated that employing a low-temperature technique in dewatering process may have positive effects on phycocyanin contents of *S. platensis*.

We found that myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), stearidonic acid (C18:4), and docosahexaenoic acid (C22:6) were the most abundant fatty acids in all studied samples. Nonetheless, different drying methods affected the long chain unsaturated FAs, whereas OD and SD methods lead to loss of PUFAs levels. On the other hand, FD played an important contribution in resistance of PUFAs content. PUFAs are more susceptible to oxidation and rapidly lost under the high temperature conditions **(Oehrl *et al*., 2001; Widjaja *et al*., 2009)**. Also, **Balasubramanian *et al*. (2013)** found that freeze-dried microalgae had the lowest value of free FAs. Therefore, our results indicated that the oven and sun drying methods of *I. galbana* are more suitable for biofuel production due to the highest content of SFAs. However, freeze drying was the best procedure to maintain MUFAs and PUFAs contents and proper for nutritional purposes.

CONCLUSION

We found that different drying methods had significant impact on some chemical and biochemical compositions, particularly fatty acids profile of *I. galbana*. The freeze-dried samples showed the highest level of protein, lipid, Chl *a*, *b*, and carotenoids, whereas the lowest levels were measured in sun drying method. Also, our findings highlight that different drying methods not only could cause a different fat yield, but also could affect the total fatty acid production. The sun-dried biomass showed the highest content of SFAs, including C14:0 and C16:0, and the maximum level of MUFAs and PUFAs were obtained in the freeze-dried samples. The results confirmed that freeze drying method can provide a better preservation method compared to other dehydration methods with the lowest nutrient lost in *I. galbana*.

Conflict of interest: The authors declare that they have no conflict of interest.

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